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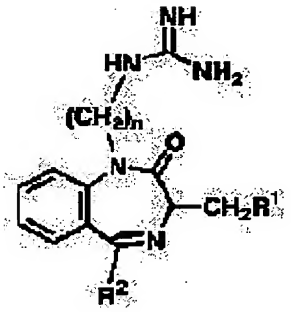
(54) BENZODIAZEPINE DERIVATIVE

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain a medicine having excellent affinity for thrombopoietin receptor and agonist activity on the receptor and having platelet production regulating action.
SOLUTION: This benzodiazepine derivative represented by the formula [R1 is phenyl group which may have a substituent group or 1H-indolyl group which may have a substituent group; R2 is a phenyl group which may have a substituent group or a lower alkyl group; (n) represents an integer of 1-4] or its pharmacologically acceptable salt has excellent affinity for thrombopoietin receptor and agonist activity on the receptor and is extremely useful as a medicine having platelet regulating action.



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* NOTICES *

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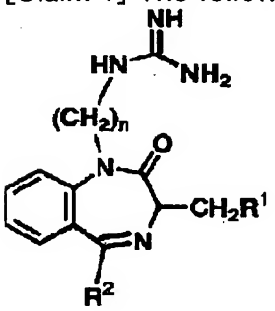
CLAIMS

[Claim(s)]
[Claim 1] The following general formula [** 1]



(R1 expresses among a formula the 1H-indolyl radical which may have the phenyl group which may have a substituent, or a substituent, R2 expresses the phenyl group or low-grade alkyl group which may have a substituent, and n expresses the integer of 1-4.) The benzodiazepine derivative shown or its salt which can be permitted in pharmacology.

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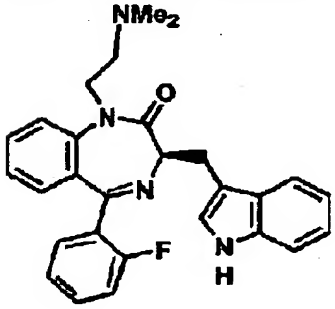
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention has the compatibility and agonist activity over the thrombopoietin receptor deeply concerned with megakaryocyte hemopoiesis and thrombopoiesis, and relates to a new benzodiazepine derivative with thrombopoiesis accommodation, or its salt which can be permitted in pharmacology.
[0002]
[Description of the Prior Art] A platelet is a blood concreteness component which plays main roles in a living body's hemostasis and thrombosis. Although the platelet was emitted into blood from the megakaryocyte which specialized and matured and was produced from the myeloid stem cell in bone marrow from the megakaryocyte precursor cell, the life is about ten days and it was known that the number will maintain a fixed value over a long period of time. Cloning of the gene of the thrombopoietin which are the main factors in the process of this megakaryocyte hemopoiesis is carried out recently. [Nature (Nature), 369 A volume and 533 Page (1994)] and thrombopoietin are c-mpl. It is the ligand of the protein (thrombopoietin receptor: MPL) which is carrying out the code. Growth and differentiation maturation of a megakaryocyte cell were stimulated from the megakaryocyte precursor cell, and it became clear that thrombopoiesis was also made to increase further [Nature, 369 volume, and 568 page (1994)]. Moreover, STAT5 which is an intracellular signaling factor when thrombopoietin combines with that receptor It also becomes clear that it activates and it is [brad (Blood), 89 volumes, and 483. Page (1997)] and this STAT5 It is surmised that gene expression required for differentiation of megakaryocyte is guided.
[0003] As a physiological active substance which adjusts thrombopoiesis through a thrombopoietin receptor to current, the low-molecular peptide currently indicated by others, WO 96/No. 40189, and WO 96/No. 40750 specification is known. [thrombopoietin / itself]
[0004] Moreover, as the benzodiazepine derivative concerning this invention, and a compound which has similar structure, it is a degree type [** 2].



Come out and the (R)-1-(2-dimethylaminoethyl)-5-(2-fluoro phenyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-2H-1, and 4-benzodiazepine-2-ON shown is known. JP,61-63666,A, JP,63-238069,A and Journal of Medicinal Chemistry (Journal of Medicinal Chemistry), Although it is indicated as a CCK antagonist in 30 volumes, 1229 etc. pages (1987), etc. and is indicated as an

antiarrhythmic by potassium ion cutoff in WO 95/No. 14470 The thrombopoietin receptor compatibility and agonist activity concerning this invention are not touched on at all by these reference.

[0005]

[Problem(s) to be Solved by the Invention] Physiological active substances, such as the above-mentioned thrombopoietin and a low-molecular peptide, adjust thrombopoiesis through a thrombopoietin receptor, and are expected as drugs which were excellent to the symptoms of the various hemopathies accompanied by reduction in a platelet count. However, although thrombopoietin is polypeptide cytokine which consists of 332 amino acid, is predicted to be decomposed within an alimentary canal and can be used as injections when using as drugs, it is considered not to be practical as internal use pharmaceutical preparation. Moreover, it has the thrombopoietin receptor compatibility and agonist activity in which the low-molecular peptide which has a thrombopoietin Mr. operation was also excellent from the possibility of internal use being an unknown etc., and development of the low-molecular non-peptide compound which can be administered orally is desired.

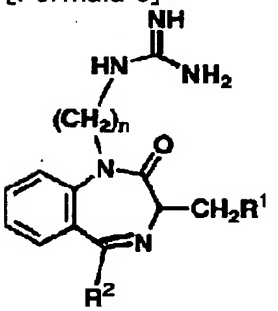
[0006] The technical problem of this invention has the outstanding thrombopoietin receptor compatibility and agonist activity, and finds out the low-molecular non-peptide compound which can be administered orally, and is to offer the remedy which can expect the effectiveness which was excellent to the various symptoms accompanied by reduction in a platelet count.

[0007]

[Means for Solving the Problem] The new benzodiazepine derivative concerning this invention or its salt which can be permitted in pharmacology finds out having the outstanding thrombopoietin receptor compatibility and agonist activity, and this invention persons came to complete this invention, as a result of repeating research wholeheartedly that said technical problem should be solved.

[0008] That is, this invention is the following general formula (I).

[Formula 3]



(I)

(-- R1 expresses among a formula the 1H-indolyl radical which may have the phenyl group which may have a substituent, or a substituent, R2 expresses the phenyl group or low-grade alkyl group which may have a substituent, and n expresses the integer of 1-4.) -- the new benzodiazepine derivative shown or its salt which can be permitted in pharmacology is offered.

[0009]

[Embodiment of the Invention] It sets to said general formula (I) of this invention, and is R1. The phenyl group shown or a 1H-indolyl radical, and R2 The phenyl group shown may have the substituent suitably and halogen atoms, such as low-grade alkyl groups, such as a methyl group, an ethyl group, and n-propyl group, a fluorine atom, a chlorine atom, and a bromine atom, a hydroxyl group, a cyano group, a nitro group, etc. are mentioned as a substituent, for example. R2 As a low-grade alkyl group shown, they are a methyl group, an ethyl group, n-propyl group, an isopropyl group, n-butyl, an isobutyl radical, and tert, for example. - Butyl, n-pentyl radical, n-hexyl group, etc. are mentioned.

[0010] Although the isomer based on dissymmetry exists in the compound shown by said general formula (I) of this invention, such isomer and its mixture are also included by the range of this invention.

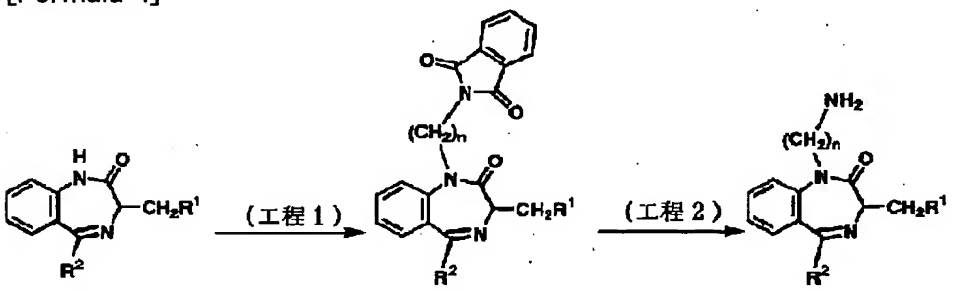
[0011] Although the compound shown by said general formula (I) of this invention or its salt

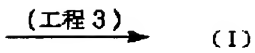
which can be permitted in pharmacology can exist as crystal form of arbitration according to manufacture conditions and it can also exist as a hydrate of arbitration, these crystal form, and a hydrate and its mixture are also included by the range of this invention. Moreover, although it may exist as solvate containing organic solvents, such as an acetone, ethanol, and a tetrahydrofuran, each matter of these gestalten is included by the range of this invention.
[0012] Changing into the salt which can be permitted in pharmacology according to a request can also change into the free base the compound shown by said general formula (I) of this invention from the generated salt. As a salt which can be permitted like pharmacology of this invention, it is mineral-acid salts, such as a hydrochloric acid, a hydrobromic acid, a hydroiodic acid, a sulfuric acid, a nitric acid, and phosphoric acid, or an acetic acid, a maleic acid, a fumaric acid, a citric acid, oxalic acid, a succinic acid, a tartaric acid, a malic acid, mandelic acid, methansulfonic acid, p-toluenesulfonic acid, and 10, for example. - Organic-acid salts, such as a camphor sulfonic acid, etc. are mentioned.
[0013] As a desirable mode of the benzodiazepine derivative concerning this invention, although the following compound and its salt which can be permitted in pharmacology can be mentioned, this invention is not limited to these examples.
(1) **) -1- (Guanidino methyl) -1, 3-dihydro-5-phenyl-3- (phenylmethyl) -2H-1 and 4-benzodiazepine-2-ON (2) (**)-1-(2-guanidino ethyl)-1, 3-dihydro-5-phenyl-3-(phenylmethyl)-2H-1, and 4-benzodiazepine-2-ON (3) () **) -1- (3-guanidino propyl) -1, 3-dihydro-5-phenyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON (4) (**)-1-(4-guanidino butyl)-1, 3-dihydro-5-phenyl-3- (phenylmethyl) -2H-1 and 4-benzodiazepine-2-ON (5) (R)-1-(4-guanidino butyl)-1, 3-dihydro-5-phenyl-3-(phenylmethyl)-2H-1, and 4-benzodiazepine-2-ON (6) () S) -1- (4-guanidino butyl) -1, 3-dihydro-5-phenyl-3- (phenylmethyl) -2H-1 and 4-benzodiazepine-2-ON (7) (**)-1-(4-guanidino butyl)-1, 3-dihydro-3-(4-hydroxy phenylmethyl)-5-phenyl-2H-1, and 4-benzodiazepine-2-ON (8) () **) -5- (2-fluoro phenyl)-1- (4-guanidino butyl) -1, 3-dihydro-3-(phenylmethyl) -2H-1 and 4-benzodiazepine-2-ON (9) (**)-1-(guanidino methyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-phenyl-2H-1, and 4-benzodiazepine-2-ON (10) () **) -1- (2-guanidino ethyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON (11) (**)-1-(3-guanidino propyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON (12) () **) -1- (4-guanidino butyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON (13) (R)-1-(4-guanidino butyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON (14) () S) -1- (4-guanidino butyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON (15) (**)-1-(4-guanidino butyl)-1, 3-dihydro-3-(5-methyl-1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON (16) () **) -5- (2-fluoro phenyl)-1- (4-guanidino butyl) -1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-2H-1 and 4-benzodiazepine-2-ON (17) (**)-1-(guanidino methyl)-1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, and 4-benzodiazepine-2-ON (18) () **) -1- (2-guanidino ethyl) -1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON (19) (**)-1-(3-guanidino propyl)-1, 3-dihydro-5-methyl-3- (Phenylmethyl) -2H-1, 4-benzodiazepine-2-ON (20) (**)-1-(4-guanidino butyl)-1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON [0014] (21) R) -1- (4-guanidino butyl) -1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON (22) (S)-1-(4-guanidino butyl)-1, 3-dihydro-5-methyl-3- (phenylmethyl) -2H-1 and 4-benzodiazepine-2-ON (23) (**)-1-(4-guanidino butyl)-1, 3-dihydro-3-(4-hydroxy phenylmethyl)-5-methyl-2H-1, and 4-benzodiazepine-2-ON (24) () **) -1- (4-guanidino butyl) -1, 3-dihydro-5-isopropyl-3- (phenylmethyl) -2H-1 and 4-benzodiazepine-2-ON (25) (**)-1-(guanidino methyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2H-1, and 4-benzodiazepine-2-ON (26) () **) -1- (2-guanidino ethyl) -1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzodiazepine-2-ON (27) (**)-1-(3-guanidino propyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzodiazepine-2-ON (28) () **) -1- (4-guanidino butyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzodiazepine-2-ON (29) (R)-1-(4-guanidino butyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzodiazepine-2-ON (30) (S)-1-(4-guanidino butyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzo diazepine-2-ON (31) (**)-1-(4-guanidino butyl)-1, 3-dihydro-5-methyl-3-(5-methyl-1H-Indore-3-ylmethyl)-2H-1, and 4-benzodiazepine-2-ON

(32) (**)-1-(4-guanidino butyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-isopropyl-2H-1, 4-benzodiazepine-2-ON [0015] Although the compound shown by said general formula (I) of this invention can be manufactured by the following approaches, the manufacture approach of the compound concerned is not necessarily limited to this approach.

[0016]

[Formula 4]

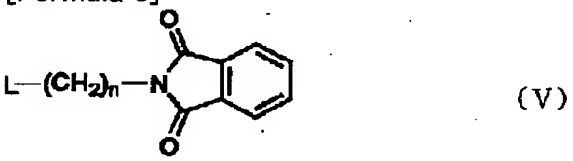




(R1, R2, and n express the above-mentioned and this meaning among a formula.)

[0017] That is, it sets at a process 1 and is JP,61-63666,A, The compound of the general formula (II) currently indicated by JP,63-238069,A and Journal of Medicinal Chemistry, 30 volumes, 1229 etc. pages (1987), etc., and the following general formula (V)

[Formula 5]



(L expresses leaving groups, such as halogen atoms, such as a chlorine atom and a bromine atom, or a mesyl oxy-radical, and n expresses the above-mentioned and this meaning.) -- compound shown N.N-dimethylformamide making it react in the range from 0 degree C to the reflux temperature of a solvent under existence of bases, such as sodium hydride and a lithium diisopropyl amide, among solvents, such as a tetrahydrofuran, -- general formula (III) A compound can be obtained.

[0018] In a process 2, the compound of a general formula (IV) can be obtained by making the compound of a general formula (III) react among solvents, such as ethanol, in hydrazine hydrate or monomethylamine, and the range from 0 degree C to the reflux temperature of a solvent.

[0019] In a process 3, the compound of said general formula (I) concerning this invention can be obtained by making the compound of a general formula (IV), and guanyl-ized reagents, such as 1H-pyrazole-1-cull BOKISAMIJIN, react in the range from 0 degree C to the reflux temperature of a solvent among solvents, such as N.N-dimethylformamide.

[0020] Thus, the physic which contains at least one of the new benzodiazepine derivative shown by said general formula (I) manufactured or the salt of its which can be permitted in pharmacology as an active principle is usually prescribed for the patient as oral agents, such as a capsule, a tablet, a fine grain agent, a granule, powder, and syrups, or injections. These pharmaceutical preparation can add the additive which can be permitted pharmacology-wise and in galenical pharmacy, and can manufacture it with a conventional method. If it is in an oral agent, namely, an excipient (a lactose, D-mannitol, corn starch, crystalline cellulose, etc.),

Disintegrator (a carboxymethyl cellulose, carboxymethyl-cellulose calcium, etc.), A binder (hydroxypropylcellulose, the hydroxypropyl methylcellulose, polyvinyl pyrrolidone, etc.), Lubricant (magnesium stearate, talc, etc.), a coating agent (the hydroxypropyl methylcellulose, white soft sugar, titanium oxide, etc.), if components for pharmaceutical preparation (polyethylene glycol etc.), such as a plasticizer, are in injections -- aquosity or business -- the time -- a dissolution mold pharmaceutical form -- constituting -- obtaining -- a resolvent -- or -- solubilizing agents (distilled water for injection, a physiological saline, propylene glycol, etc.) -- PH regulator (inorganic or organic an acid or a base), Isotonizing agents (salt, grape sugar, glycerol, etc.), Pharmaceutical preparation components, such as a stabilizing agent, are used. [0021] In an adult, it can divide into 1-2000mg by internal use, 1-200mg can be divided into 1 time per or several times day by parenteral administration, and the dose to the therapy patient of this invention compound can usually be prescribed for the patient, although it changes with a patient's symptom, age, etc.

[0022]

[Example] Hereafter, although an example explains this invention, this invention is not limited to the specific details of these examples.

[0023] Example 1 (**) -1- (4-guanidino butyl) -1, 3-dihydro-5-phenyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON and hydrochloride a(**)-N-[4-[2, 3-dihydro-2-oxo--5-phenyl-3-(Phenylmethyl) -1H-1, 4-benzodiazepine-1-IRU] butyl] phthalimide (**)-1, 3-dihydro-5-phenyl-3-(phenylmethyl)-2H-1, and 4-benzodiazepine-2-ON 4.00g (12mmol) And 0.54g (14mmol) of sodium hydride was added to the mixture of 50ml of N.N-dimethylformamide bottom 60% of ice-cooling. N-(4-BUROMO butyl) phthalimide 7.00g (25mmol) was added after bottom 1.5-hour stirring of ice-cooling, and it stirred at the room temperature for 18 hours. 100ml of water was added to the reaction mixture, and suction **** of the solvent was carried out. Residue was melted to ethyl acetate, sequential washing was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (silica gel, hexane:ethyl acetate = 2:1) refined residue, and the yellow amorphous solid-state was obtained. Diisopropyl ether is added to the obtained solid-state, it was crystallized, suction filtration was carried out, and 5.90g (91% of yield) of fine yellow crystals with a melting point of 124-126 degrees C was obtained.

elemental-analysis value 3OC34H29N3 theoretical value C and 77.40; H and 5.54; N and 7.96 experimental values C and 77.08; H, 5.52; N, 7.94b (**) -1- (4-amino butyl) -1, 3-dihydro-5-phenyl-3- (Phenylmethyl) -2H-1, 4-benzodiazepine - 2-ON (**)-N-[4-[2, 3-dihydro-2-oxo--5-phenyl-3-(phenylmethyl)-1H-1, and 4-benzodiazepine-1-IRU] butyl] phthalimide 4.00g (7.6mmol), The heating reflux of the 0.41ml [of hydrazine hydrate] (8.5mmol) and ethanol 40ml mixture was carried out for 4 hours. 100ml of sodium-hydroxide water solutions was added to the reaction mixture 5% after radiationnal cooling, and ethyl acetate extracted. Sequential washing of the organic layer was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (silica gel, a dichloromethane:methanol = 5:1) refined residue, and 1.17g (39% of yield) of fine yellow amorphous solid-states was obtained.

IR spectrum nu (liq) cm-1 : 3376, 1676, 1606NMR spectrum delta (CDCl3) ppm : 1.21-1.35 (2H, m), 1.40-1.60 (4H, m), 2.48-2.57 (2H, m), 3.59 (2H, d, J= 6.5Hz) 3.65 (1H, ddd, J= 13.5, 7.5, 5.5Hz), 3.78 (1H, t, J= 6.5Hz) 4.43 (1H, dt, J = 14 or 7.5Hz), 7.15-7.56 High-resolution mass spectrum : C26H27N3 (14H, m) O theoretical value m/z : 397.2154 experimental values m/z : 397.2150c (**) -1- (4-guanidino butyl) -1, 3-dihydro-5-phenyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON and hydrochloride (**)-1-(4-amino butyl)-1, 3-dihydro-5-phenyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON 1.50g (3.8mmol) It added to 1H-pyrazole-1-cull BOKISAMIJIN and 0.55g [of hydrochlorides] (3.8mmol), N, and N-diisopropyl ethylamine 0.49g (3.8mmol), and the mixture of 6ml of N.N-dimethylformamide. Suction filtration of the diethylether 50ml was added and carried out to the reaction mixture after 4-hour stirring at the room temperature. The column chromatography (silica gel, a dichloromethane:methanol = 5:1) refined residue, and 1.27g (68% of yield) of fine yellow amorphous solid-states was obtained.

Elemental-analysis value C27H29N5 O-HCl and 5/4 H2 O theoretical value C, 65.05; H, 6.57; N,

14.05 experimental values C, 65.04; H, 6.61; N, 14.10 [0024] Example 2 (**) -1- (4-guanidino butyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON and hydrochloride a(**)-N-[4-[2, 3-dihydro-3-(1H-Indore-3-ylmethyl)-2-oxo--5-phenyl-1H-1, 4-benzodiazepine-1-IRU] butyl] phthalimide (**) -1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-phenyl-2H-1, and 4-benzodiazepine-2-ON 1.50g (4.1mmol) And 0.17g (4.3mmol) of sodium hydride was added to the mixture of 30ml of N.N-dimethylformamide bottom 60% of ice-cooling. N-(4-BUROMO butyl) phthalimide 2.48g (8.8mmol) was added after bottom 1-hour stirring of ice-cooling, and it stirred at the room temperature for 16 hours. 100ml of water was added to the reaction mixture, and suction **** of the solvent was carried out. Residue was melted to ethyl acetate, sequential washing was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (silica gel, a dichloromethane:methanol = 50:1) refined residue, and 2.13g (92% of yield) of yellow amorphous solid-states was obtained.
elemental-analysis value 4OC36H30N3 theoretical value C and 76.31; H and 5.34; N and 9.89 experimental values C and 76.18; H, 5.16; N, 9.85b (**) -1- (4-amino butyl) -1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON (**)-N-[4-[2, 3-dihydro-3-(1H-Indore-3-ylmethyl)-2-oxo--5-phenyl-1H-1, The heating reflux of the 4-benzodiazepine-1-IRU] butyl] phthalimide 1.50g (2.7mmol), 0.14ml [of hydrazine hydrate] (2.9mmol), and ethanol 20ml mixture was carried out for 5 hours. 50ml of sodium-hydroxide water solutions was added to the reaction mixture 5% after radiationnal cooling, and ethyl acetate extracted. Sequential washing of the organic layer was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (an alumina, a dichloromethane:methanol = 20:1->9:1) refined residue, and 0.81g (70% of yield) of fine brown amorphous solid-states was obtained.
IR spectrum nu (KBr) cm-1 : 3360, 1672, 1602NMR spectrum delta (CDCl3) ppm : 1.22-1.57 (6H, m), 2.53 (2H, dd, J= 13.5, 6.5Hz) 3.63-3.71 (2H, m), 3.78- 3.84 (2H, m) and 4.44 (1H, dt, J = 14 or 7Hz) -- 7.05-7.65 (14H, m), 8.01 High-resolution mass spectrum : C28H28N4 (1H, brs) O theoretical value m/z : 436.2263 experimental values m/z : 436.2263c (**) -1- (4-guanidino butyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON and hydrochloride (**)-1-(4-amino butyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-phenyl-2H-1, 4-benzodiazepine-2-ON 0.81g (1.9mmol) It added to 1H-pyrazole-1-cull BOKISAMIJIN and 0.27g [of hydrochlorides] (1.9mmol), N, and N-diisopropyl ethylamine 0.24g (1.9mmol), and the mixture of 1.8ml of N.N-dimethylformamide. Suction filtration of the diethylether 20ml was added and carried out to the reaction mixture after 2.5-hour stirring at the room temperature. The column chromatography (silica gel, a dichloromethane:methanol = 5:1) refined residue, and 0.55g (56% of yield) of light yellow amorphous solid-states was obtained.
Elemental-analysis value C29H30N6 O-HCl and 1/2 H2 O theoretical value C, 66.46; H, 6.15; N, 16.04 experimental values C, 66.12; H, 6.28; N, 15.68 [0025] Example 3 (**) -1- (4-guanidino butyl) -1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON and hydrochloride a(**)-N-[4-[2, 3-dihydro-5-methyl-2-oxo--3-(phenylmethyl)-1H-1, 4-benzodiazepine-1-IRU] butyl] phthalimide (**) into -1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, and 4-benzodiazepine-2-ON 2.65g (10mmol) and the mixture of 50ml of N.N-dimethylformamide 0.42g (11mmol) of sodium hydride was added bottom 60% of ice-cooling. N-(4-BUROMO butyl) phthalimide 7.00g (25mmol) was added after bottom 1-hour stirring of ice-cooling, and it stirred at the room temperature for 4 hours. 150ml of water was added to the reaction mixture, and suction **** of the solvent was carried out. Residue was melted to ethyl acetate, sequential washing was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (silica gel, hexane:ethyl acetate = 2:1) refined residue, and the colorless amorphous solid-state was obtained. Diisopropyl ether is added to the obtained solid-state, it was crystallized, suction filtration was carried out, and 3.75g (81% of yield) of colorless crystals with a melting point of 137-139.5 degrees C was obtained.
elemental-analysis value 3OC29H27N3 theoretical value C and 74.82; H and 5.85; N and 9.03 experimental values C and 75.06; H, 6.06; N, 9.07b (**) -1- (4-amino butyl) -1, 3-dihydro-5-

methyl-3- (Phenylmethyl) -2H-1, 4-benzodiazepine - 2-ON (**)-N-[4-[2, 3-dihydro-5-methyl-2-oxo--3-(phenylmethyl)-1H-1, and 4-benzodiazepine-1-IRU] butyl] phthalimide 3.65g (7.8mmol), The heating reflux of the 0.42ml [of hydrazine hydrate] (8.7mmol) and ethanol 50ml mixture was carried out for 3.5 hours. 100ml of sodium-hydroxide water solutions was added to the reaction mixture 5% after radiationnal cooling, and ethyl acetate extracted. Sequential washing of the organic layer was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (silica gel, a dichloromethane:methanol = 5:1) refined residue, and 2.00g (76% of yield) of brown oil was obtained.
IR spectrum nu (liq) cm-1 : 3368, 1674, 1628NMR spectrum delta (CDCl3) ppm : 1.24-1.60 (4H, m), 1.63 (2H, brs), 2.46 (3H, s), 2.61 (2H, t, J= 7.5Hz), 3.30-3.35 (1H, m), 3.59-3.66 (3H, m), 4.27 (1H, dt, J = 14 or 7Hz), 7.13-7.51 High-resolution mass spectrum : C21H25N3 (9H, m) O theoretical value m/z : 335.1998 experimental values m/z : 335.1991c (**) -1- (4-guanidino butyl) -1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON and hydrochloride (**)-1-(4-amino butyl)-1, 3-dihydro-5-methyl-3-(phenylmethyl)-2H-1, 4-benzodiazepine-2-ON 1.50g (4.5mmol) It added to 1H-pyrazole-1-cull BOKISAMIJIN and 0.66g [of hydrochlorides] (4.5mmol), N, and N-diisopropyl ethylamine 0.78ml (4.5mmol), and the mixture of 4.5ml of N.N-dimethylformamide. Suction filtration of the diethylether 45ml was added and carried out to the reaction mixture after 4-hour stirring at the room temperature. The column chromatography (silica gel, a dichloromethane:methanol = 5:1) refined residue, and 0.87g (45% of yield) of light brown amorphous solid-states was obtained.
Elemental-analysis value C22H27N5 O-HCl-H2 O theoretical value C, 61.17; H, 7.00; N, 16.21 experimental values C, 61.00; H, 7.02; N, 16.13 [0026] Example 4 (**) -1- (4-guanidino butyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzodiazepine-2-ON and hydrochloride a(**)-N-[4-[2, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2-oxo--1H-1, 4-benzodiazepine-1-IRU] butyl] phthalimide (**) -1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2H-1, and 4-benzodiazepine-2-ON 3.00g (9.9mmol) And 0.42g (11mmol) of sodium hydride was added to the mixture of 30ml of N.N-dimethylformamide bottom 60% of ice-cooling. N-(4-BUROMO butyl) phthalimide 7.00g (25mmol) was added after bottom 1.5-hour stirring of ice-cooling, and it stirred at the room temperature for 3 hours. 150ml of water was added to the reaction mixture, and suction **** of the solvent was carried out. Residue was melted to ethyl acetate, sequential washing was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (silica gel, a dichloromethane -> dichloromethane:methanol = 20:1) refined residue, and the yellow amorphous solid-state was obtained. Diisopropyl ether is added to the obtained solid-state, it was crystallized, suction filtration was carried out, and 3.30g (66% of yield) of colorless crystals with a melting point of 171.5-173.5 degrees C was obtained.
elemental-analysis value 4OC31H28N3 theoretical value C and 73.79; H and 5.59; N and 11.10 experimental values C and 73.76; H, 5.66; N, 11.03b (**) -1- (4-amino butyl) -1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzodiazepine-2-ON (**)-N-[4-[2, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-2-oxo--1H-1, The heating reflux of the 4-benzodiazepine-1-IRU] butyl] phthalimide 3.30g (6.5mmol), 0.35ml [of hydrazine hydrate] (7.2mmol), and ethanol 40ml mixture was carried out for 5 hours. 100ml of sodium-hydroxide water solutions was added to the reaction mixture 5% after radiationnal cooling, and ethyl acetate extracted. Sequential washing of the organic layer was carried out with water and saturation brine, and reduced pressure distilling off of the solvent was carried out after desiccation with the sodium sulfate. The column chromatography (an alumina, a dichloromethane:methanol = 10:1) refined 1.20g of 2.70g of residue, and 0.45g (18% of yield) of colorless amorphous solid-states was obtained.
IR spectrum nu (liq) cm-1 : 3304, 1668, 1626NMR spectrum delta (CDCl3) ppm : 1.25-1.60 (6H, m), 2.47 (3H, s), 2.62 (2H, t, J= 7Hz), 3.44 (1H, dd, J= 14.5, 6Hz), 3.60-3.67 (2H, m), 3.78-3.83 (1H, m), 4.23-4.29 (1H, m), 7.00-7.56 (9H, m), 8.07 High-resolution mass spectrum : C23H26N4 (1H, brs) O theoretical value m/z: 374.2107 experimental values m/z : 374.2104c (**) -1- (4-guanidino butyl) -1, 3-dihydro-3- (1H-Indore-3-ylmethyl)-5-methyl-2H-1, 4-benzodiazepine-2-ON and hydrochloride (**)-1-(4-amino butyl)-1, 3-dihydro-3-(1H-Indore-3-ylmethyl)-5-methyl-

2H-1, 4-benzodiazepine-2-ON 1.50g (4.0mmol) It added to 1H-pyrazole-1-cull BOKISAMIJIN and 0.59g [of hydrochlorides] (4.0mmol), N, and N-diisopropyl ethylamine 0.70ml (4.0mmol), and the mixture of 4ml of N.N-dimethylformamide. Suction filtration of the diethylether 40ml was added and carried out to the reaction mixture after 15-hour stirring at the room temperature. The column chromatography (silica gel, a dichloromethane:methanol = 5:1) refined residue, and 0.50g (25% of yield) of light yellow amorphous solid-states was obtained.
Elemental-analysis value C24H28N6 O-HCl and 9/4 H2 O theoretical value C, 58.41; H, 6.84; N and 17.03 Experimental value C, 58.14; H, 6.51; N and 16.85 [0027] Hereafter, in order to evaluate the thrombopoietin receptor binding affinity of this invention compound, the contention experiment to the thrombopoietin receptor of thrombopoietin and a test compound was conducted. Moreover, in order to check the agonist activity over the thrombopoietin receptor of this invention compound, it is an intracellular signaling factor through a thrombopoietin receptor. Activation of STAT5 was evaluated using the gel shift assay method.
[0028] Construction of an example of trial 1 Homo-sapiens thrombopoietin receptor (MPL) manifestation plasmid (1) The phage clone holding all the fields of MPL cDNA was first obtained by the plaque hybridization method. To this sake Some Homo sapiens MPL cDNA was acquired from the Homo sapiens embryo liver cDNA (product made from CLONTECH) by the PCR method. In addition, for the initiation codon of MPL cDNA to a termination codon, the array of the upstream of an initiation codon is EMBL X73551 to GenBank M90102. It is registered. A of the primer for PCR, and the initiation codon of MPL from -- counting -- from a 331 base eye the sense primer 5 based on the array of a 350 base eye -- 'antisense primer 5'-TCAAGGCTGCTGCCAATAGC-3 based on the array of a 1888 base eye to -GTGCGTCTCTTCTTTCCGCT-3' and a 1907 base eye' was used. Takara EX Taq (TAKARA SHUZO CO., LTD. make) performed PCR on condition that usual using the attached reaction buffer. this -- an PCR product -- after agarose gel electrophoresis and from gel According to the attached protocol, it collected using SUPREC-01 (TAKARA SHUZO CO., LTD. make). It collected. Using Rediprime DNA labelling system (product made from Amersham), according to the attached protocol, the label of the PCR product was carried out by [alpha-32P] dCTP, and it was used as the probe. According to the attached protocol, all the coding fields of MPL cDNA and the phage clone which holds 60 or more bases of upstream from an initiation codon at least were isolated from Human Fetal Liver 5'-STRETCH cDNAlibrary using this (product made from CLONTECH), and phage was prepared according to the conventional method.
(2) To a degree By the PCR method, he is Homo sapiens MPL. The code of the extracellular field cDNA (1 to 491 position amino acid sequence) is carried out. DNA was acquired. the phage from which the mold for PCR was obtained above -- using -- primer the sense primer 5 based on the array for 17 bases from 28 base upstream of the initiation codon of MPL -- '-CTAAGGCAGGCACACAG-3' 486 from -- Antisense primer 5'-GGTGACCCAGGCGGTCTCGGTGGC-3' based on the 491st amino acid sequence was used. Under the present circumstances, a BstEII site is put in so that the C terminal field of MPL extracellular field protein can connect with Homo sapiens IgG Fc, and it was made further in agreement [a reading frame]. moreover, the Homo sapiens IgG Fc field cDNA -- B.D.Bennett ** -- reference [Journal of Biological Chemistry (Journal of Biological Chemistry) -- 266 volumes and 23060 It refers to page (1991)] and is sense primer 5'-CGCGGTCACCGACAAAACTCA-3'. Antisense primer 5'-GCACTCATTTACCCGGAGACAGGGAGA-3' It uses. It is made from QUICK-CLONE cDNA (product made from CLONTECH) of a Homo sapiens spleen, It acquired by the PCR method. Thus, it was obtained. According to the process which describes an PCR product below, the nest and the MPL manifestation plasmid were built to pCR3 (product made from Invitrogen).
(3) It was obtained by PCR. Escherichia coli TOP 10 after inserting the MPL extracellular field cDNA and the Homo sapiens IgG Fc field cDNA in a pCR3 breast-feeding cell expression vector according to an attached protocol using EUKARYOTIC TA CLONING KIT (product made from Invitrogen) The transformation was carried out. Mass culture of the obtained transformant was carried out according to the conventional method. From now on according to the conventional method, the plasmid was prepared, and it was named MPL(B)-pCR3 and IgG Fc(B)-pCR3,

respectively.
(4) Abbreviation 200microg MPL(B)-pCR3 BstEII of 0.64 units (Toyobo Co., Ltd. make) Agarose electrophoresis was presented with this after cutting by ScaI (TAKARA SHUZO CO., LTD. make) of 200 units. 3085 base pairs which include a MPL cDNA field from this plasmid The gel fragment containing a DNA fragment is cut down and it is a conventional method from the gel fragment. DNA was extracted.
(5) About 20microg IgG Fc(B)-pCR3 was presented after dephosphorization, and agarose electrophoresis was presented with this by alkaline phosphatase (Toyobo Co., Ltd. make) after cutting by BstEII (Toyobo Co., Ltd. make) of 40 units, and ScaI (TAKARA SHUZO CO., LTD. make) of 80 units. 4150 base pairs which include the IgG Fc field cDNA from this plasmid The gel fragment containing a DNA fragment is cut down and it is from the gel fragment. DNA was extracted.
(6) (4) It obtained. A DNA fragment (about 30 ng) and (5) It obtained. About a DNA fragment (about 20 ng), it is 4.6 units. It was made to connect in T-four DNA ligase (Toyobo Co., Ltd. make). By the electroporation method, the transformation was carried out to the Escherichia coli XL1-Blue stock (product made from Stratagene). Mass culture of the obtained transformant was carried out according to the conventional method. From now on according to the conventional method, the plasmid was prepared, and it was named MPL-IgG Fc (B)/pCR3.
[0029] Example of trial 2 Homo-sapiens IgG Fc field fusion Homo sapiens MPL Homo sapiens embryo who discovers protein (MPL-IgG) to stability Production and MPL-IgG of 293 cells At purification MPL-IgG Fc (B)/pCR3, he is a Homo sapiens embryo by the electroporation method [technical third edition [application editing [of Yoshinari Watanabe:tissue culture]] (edited by Japan Tissue Culture Association), 501 page, and 1996]. The transformation of the 293 cells was carried out. Homo sapiens embryo by whom the transformation was done 0.4 mg/ml after cultivating 293 cells for two days by the fetal-calf-serum content DMEM culture medium 10% It cultivated for about two weeks by 10% fetal-calf-serum content DMEM containing JIENETISHIN (product made from LIFE TECHNOLOGIES), and the transformant was obtained. This transformant was cultivated until it became confluence about 50%, and it exchanged for the DMEM culture medium which contains NYUTORI dahoma (product made from Boehringer Mannheim) 1%, and culture was continued. Culture was continued for four weeks from three weeks, exchanging culture media weekly [about]. After carrying out centrifugal [of this culture medium] and collecting culture supernatants, it filtered using VacuCap (product made from Gelman Sciences). From the culture supernatant of about 7 L, a column chromatography is performed according to an attached protocol using HiTrap Protein G (product made from Pharmacia-Biotech), and it is MPL-IgG. It refined.
[0030] To the contention experiment microtiter monotonous well of the thrombopoietin and the test compound using the example of trial 3ELISA method, it is 100microl. 10 ng diluted with PBS (-) MPL-IgG It covered with 4 degrees C all night. After dissolving a test compound in DMSO, using PBS(-)/1%BSA/0.05% Tween20, the thrombopoietin (R&D shrine make) solution (last concentration 0.1 nM) was mixed, and the subject was produced so that last DMSO content might become 5%. It is MPL-IgG from a well. The solution was removed, the subject was added and it covered with the room temperature for 1 hour or more. this solution is removed -- 200microl PBS (-) / after washing a well base by Tween20 0.05%, it incubated at the room temperature by goat Anti-Human TPO Neutralizing Antibody (R&D shrine make) for 1 hour or more. 200 mul Horseradish peroxidase indicator ass anti-goat PBS (-) / after washing a well by Tween20 0.05% By the IgG antibody (product made from Chemicon International), it incubated at the room temperature for 1 hour or more. 200microl 100microl after washing a well by PBS (-) which contains Tween20 0.05% The TMB solution (product made from DAKO) was added, and it incubated for 5 minutes at the room temperature. 100microl 1M H2SO4 (Wako Pure Chem make) was added, and the reaction was suspended. Association of thrombopoietin when measuring optical density in 450 nm and not adding the test compound It considered as 100%, the joint depressant action of the thrombopoietin by the test compound was investigated, and the compatibility to a thrombopoietin receptor was evaluated. A result is shown in drawing 1 . this invention compound showed the outstanding compatibility to a thrombopoietin receptor so that

clearly from this result.
[0031] Example of trial 4 Homo sapiens MPL It is discovered to stability. Production of a BaF/mpl cell (1) Phage DNA obtained in the example 1 of a trial As mold and a primer, it is MPL. Sense primer 5'-CTAAGGCAGGCACACAGTG-3' based on the array for 19 bases from 28 base upstream of an initiation codon Antisense primer 5'-TCAAGGCTGCTGCCAATAGC-3' based on the array of a 1888 base eye to a 1907 base eye was used. Thus, it was obtained. It introduces into pCR3 (product made from Invitrogen) according to the process which describes an PCR product below, and is MPL. The recombination plasmid for a manifestation was built.
(2) Using EUKARYOTIC TA CLONING KIT (product made from Invitrogen), follow an attached protocol and it is the above. It is Escherichia coli about the this recombination vector after inserting the PCR product MPL cDNA in a pCR3 breast-feeding cell expression vector. It introduced into TOP10. Mass culture of the obtained transformant was carried out according to the conventional method. From now on according to the conventional method, the plasmid was prepared. The plasmid obtained above is used and it is the mouse interleukin 3 dependency mouse pro B cell origin by the electroporation method. The transformation of the Ba/F3 cell was carried out. The transformation was carried out. They are 5 units/ml mouse interleukin 3 (IL-3, product made from Genzyme), and 50microM about Ba/F3 cell. 0.8mg/ml after cultivating for one day by fetal-calf-serum content RPMI1640 culture medium 10% containing beta-mercaptoethanol It cultivated for about two weeks by the selective medium containing JIENETISHIN (product made from LIFE TECHNOLOGIES), and the transformant was obtained. it checks that this transformant is a thrombopoietin dependency by commercial Homo sapiens thrombopoietin (R&D company make) -- it was named the BaF/mpl cell.
[0032] Preparation BaF/mpl of the nucleus extract of the cell stimulated by example of trial 5 test compound A cell or Ba/F3 It is 50micro M beta-mercaptoethanol and 0.4 mg/ml under growth factor nonexistence about a cell. After cultivating by fetal-calf-serum content RPMI1640 culture medium 10% containing JIENETISHIN for about 16 hours, a cell is settled by centrifugal, and it is 1x107. A cell/ml The cell was suspended in fetal-calf-serum content RPMI1640 culture medium 10% which contains 50micro M beta-mercaptoethanol so that it may become. This suspension 0.99 ml was cultivated further for 3 to 4 hours. A subject is 10microl to cell suspension after dissolving a test compound in DMSO. In addition, it cultivated for 15 minutes. The nucleus extract from a cell is experimental-medicine separate volume biotechnology manual UP series. It carried out according to the cytokine laboratory procedure (Yodosha, p115, 1997). That is, it is ice-cooled 8ml 0.4 mM EDTA about this cell culture liquid. 0.4 mM Na3VO4 In addition to included PBS (-), the cell was settled by centrifugal at 4 degrees C. Furthermore, buffer H of ice-cooled 0.4 ml The cell was suspended in [20 mM Hepes-NaOH (pH7.9), 1 mM EDTA, 0.1 mM EGTA, 2 mM MgCl2, 1 mM Na3VO4 20 mM NaF, 1 mM DTT (dithiothreitol), and 1 mg/ml Leupeptin], and the cell was settled by centrifugal at 4 degrees C. Furthermore, it suspended in the buffer I of 0.4 ml which ice-cooled the cell (0.2%NP-40 content buffer H), centrifugal was carried out at 4 degrees C, and precipitation was obtained. It is 20microl about precipitation. Buffer K (420 mM NaCl and 20% glycerol content buffer H) was added and stirred, and the at-long-intervals alignment was carried out at 4 degrees C for 20 minutes. This supernatant liquid was made into the nucleus extract.
[0033] Example of trial 6STAT5 Adjustment 5microl of a probe STAT5 Gel Shift Oligonucleotides (product made from Santa Cruz Biotechnology), and 1microl A polynucleotide kinase buffer (Toyobo Co., Ltd. make) and 1microl A polynucleotide kinase (the Toyobo Co., Ltd. make, 10 units/mul) and 3microl [gamma-32P] ATP (product made from Amersham) It mixed and reacted at 37 degrees C for 1 hour. Ethanol precipitation was performed after heating for about 15 minutes at 65 degrees C. 100 after drying precipitation mul It dissolved by STE (100 mM NaCl, 10 mM Tris-HCl (pH7.5), 1 mM EDTA). this solution the SEPAKORU mini column (Seikagaku make) filled up with SephadexG50 (product made from Pharmacia) suspended in STE -- applying -- several ml STE -- an interface -- adding -- every [about 0.5 ml] -- it isolated preparatively. The high fraction of radioactivity was checked by the GM counter surveymeter, and six solutions with high activity were mixed. It is this solution After ethanol precipitation and 100 mul It dissolves in water and is this. It used as STAT5 probe.

[0034] Example of trial 7 gel shift assay gel shift assay is experimental-medicine separate volume biotechnology manual UP series. Cytokine laboratory procedure (Yodosha, p115, 1997) It carried out by applying correspondingly. it adjusted in the example 5 of a trial a BaF/mpl cell -- and -- each nucleus extract 1microl of Ba/F3 cell -- 1microl STAT5 probe and 1microl 1microg/mu l poly(dI) and poly (dC), (the product made from Pharmacia-Biotech), 10microl Binding buffer (20 mM Hepes-NaOH (pH7.9), 2 mM EDTA, 0.2 % NP-40, 60 mM NaCl, 10% glycerol) And 7microl Water was added and it was left more than for 30 minutes at the room temperature. 6.7 of reaction mixture mul It applies to polyacrylamide gel 5%, and is 10 mA at 0.25xTBE (22.5 mM Tris-borate and 0.5 mM EDTA (pH8.0)). Electrophoresis was performed for about 1 hour. It dried, after dipping gel in -10% methanol solution of acetic acids 10%. Gel is contacted to an imaging plate (Type III and Fuji Photo Film Co., Ltd. make) for about 1 hour. It incorporates as an image with a BAS2000 image analyzer (Fuji Photo Film Co., Ltd. make), and is a PICT ROGURA fee (Fuji Photo Film Co., Ltd. make). It printed. A result is shown in drawing 2 . It is this invention compound so that clearly from this result. By activating STAT5 showed that the agonist activity over a thrombopoietin receptor was shown.

[0035]

[Effect of the Invention] The benzodiazepine derivative shown by said general formula (I) of this invention or its salt which can be permitted in pharmacology has the outstanding compatibility to a thrombopoietin receptor, and the agonist activity over this receptor, and is very useful as a remedy with thrombopoiesis accommodation.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing which measured the joint depressant action of the thrombopoietin of this invention compound, and evaluated the compatibility over the thrombopoietin receptor of this invention compound.
[Drawing 2] this invention compound By activating STAT5, it is drawing which evaluated that the agonist activity over a thrombopoietin receptor was shown using the gel shift assay method.

[Translation done.]

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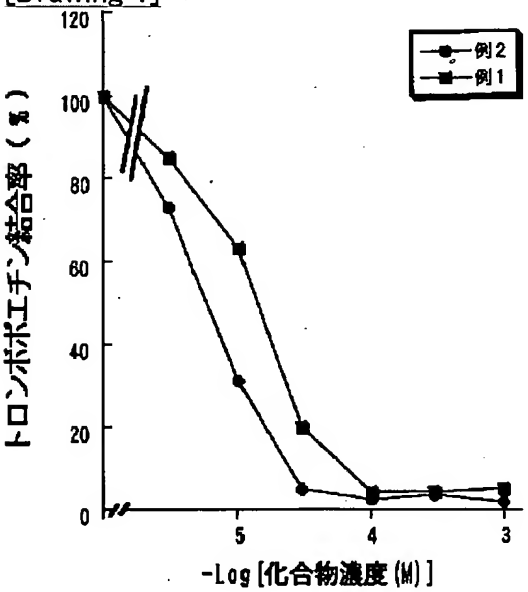
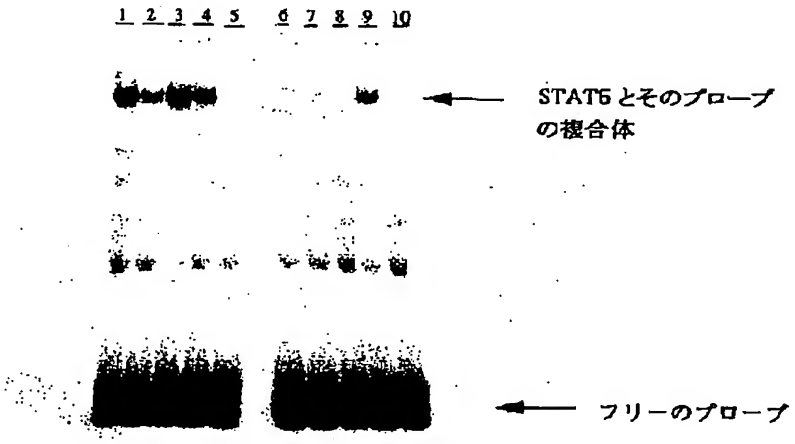
DRAWINGS

[Drawing 1]



[Drawing 2]

レーン1:100 μM の例2刺激 BaF/mpl 細胞の核抽出液+STAT5 プローブ
レーン2:100 μM の例1刺激 BaF/mpl 細胞の核抽出液+STAT5 プローブ
レーン3:20 ng のヒトトロンボポエチン刺激 BaF/mpl 細胞の核抽出液+STAT5 プローブ
レーン4:100 units のマウス IL-3 刺激 BaF/mpl 細胞の核抽出液+STAT5 プローブ
レーン5:無刺激 BaF/mpl 細胞の核抽出液+STAT5 プローブ
レーン6:100 μM の例2刺激 Ba/F3 細胞の核抽出液+STAT5 プローブ
レーン7:100 μM の例1刺激 Ba/F3 細胞の核抽出液+STAT5 プローブ
レーン8:20 ng のヒトトロンボポエチン刺激 Ba/F3 細胞の核抽出液+STAT5 プローブ
レーン9:100 units のマウス IL-3 刺激 Ba/F3 細胞の核抽出液+STAT5 プローブ
レーン10:無刺激 Ba/F3 細胞の核抽出液+STAT5 プローブ



[Translation done.]